of nucleation and control it, and in situ X-ray diffraction of crystals will be examined in this overview lecture. Conclusion: There is no universal solution in the search for good crystals. If anything, the search has become more difficult as more challenging targets are attempted. A multi-faceted approach is therefore required.

Keywords: protein crystallization strategy, automation, screening

MS.23.2

Acta Cryst. (2008). A**64**, C48

Photochemically induced nucleation of protein

Tetsuo Okutsu, Kenji Furuta, Susumu Haruta, Takashi Kuroiwa, Hiroshi Hiratsuka
Gunma University, Department of Chemistry and Chemical biology, 1-5-1 Tenjincho, Kiryu, Gunma, 376-8515, Japan, E-mail：okutsu@chem-bio.gunma-u.ac.jp

An important area of post-genomic research is the determination of 3-D protein structures. The main technique used for this purpose is X-ray crystallography. Protein crystallization experiments are carried out in the presence of crystallization agents, e.g., inorganic salts, non-adsorbing polymers, and alcohols, which reduce protein solubility and increase intermolecular interactions to form cluster, nucleus and crystal. In these experiments, account needs to be taken of the protein concentration, nature and concentration of the crystallization agent, pH, buffer constitution, and temperature. Here, we demonstrate photochemically-induced crystallization of metastable protein solutions by weak UV irradiation for several ten seconds. Intermediates, neutral radicals at tryptophan or tyrosine residual produced by one photon absorption, enhance nucleation. The radical forms protein dimmer that is detected by an SDS-PAGE electrophoresis experiment. An addition of polyethylene glycol (PEG) greatly enhances light-induced nucleation. PEG affects to shorten the intermediate radical lifetime, which suggests that PEG assists to form dimmer. We consider that the photochemical dimmer behaves as smallest cluster to grow critical nucleus. The smallest cluster formation is the rate determining step in classical nucleation theory due to surface energy disadvantage. The photochemical dimmer is formed by a covalent bond, and the nucleation is initiated from stable dimer. The nucleation enhancement is reasonably explained. The present researches results point out the development of a new method for controlling nucleation and growth that could be applied structural genomics and pharmaceutical industry for instance.

Keywords: photochemistry, protein, crystal growth

MS.23.3

Acta Cryst. (2008). A**64**, C48

Applications of designed ankyrin repeat proteins as chaperones in structural biology

Markus G. Gruetter
University of Zurich, Biochemistry, Winterthurerstr. 190, Zurich, Zurich, 8057, Switzerland, E-mail：gruetter@bioc.uzh.ch

Repeat proteins are ubiquitous protein-protein interaction molecules in biology [1]. We made use of this feature in vitro and designed ankyrin repeat proteins (DARPins) which consist of repeat modules with fixed framework residues and randomized surface residues suitable for target binding. The random assembly of such modules yields combinatorial libraries of DARPins of varying length and large diversities. DARPins are very stable, soluble and produced in large amounts by bacterial expression. By using ribosome display highly specific binders against different protein targets with low nanomolar affinity can be selected. This opens the possibility to crystallize a target protein in complex with a variety of DARPins and therefore enhances the chance of obtaining structures of target proteins that are difficult to crystallize. We selected DARPins having high affinity and specificity for proteases, kinases and membrane proteins and used them for cocrystallization of the target protein. The methodology and the X-ray structures of a DARPin-MBP- [2], a DARPin-kinase- [3], a DARPin-caspase-2- [4] and a DARPin-membrane protein-complex [5] have been published. This illustrates the usefulness of this novel technology in structural biology. It opens a new avenue in macromolecular crystallization and is an attractive alternative to antibodies for the crystallization of membrane proteins. In addition, highly specific DARPin binders and/or inhibitors of target proteins allow the analysis of the role of this protein in signalling pathways.

〔1〕Forrer, P., et al. FEBS Lett,2003.539,2-6.
〔2〕Binz, H.K., et al. Nat Biotechnol,2004.22,575-82.
〔3〕Kohl, A., et al. Structure, 2005.13,1131-41.
〔4〕Schweizer, A., et al. Structure, 2007.15,625-636.
〔5〕Sennhauser, G., et al. PLoS Biology, 2007.5,e7.

Keywords: crystallization, designed proteins, membrane proteins

MS.23.4

Acta Cryst. (2008). A**64**, C48

A simple method to introduce anomalous scatterers in a wide number of proteins

Jakoncic Jean, Vivian Stojanoff
Brookhaven National Laboratory, National Synchrtron Light Source, BNL BLDG 725D NSLS, Upton, NY, 11973, USA, E-mail：jakoncic@bnl.gov

A recent Protein Data Bank survey indicated that more than 5500 X-ray crystal structures contain at least one Sulfate ion and the total number of X-ray crystal structures is approximately 43000 therefore approximately 13 % of the total structures contain Sulfate. Since Sulfate ions are predominantly introduced during the crystallization step, we have taken advantage of a simple substitution in the crystallization reagent. Sulfate (SO_4) was substituted with Selenate (SeO_4) during the crystallization of two model proteins known to crystallize in SO_4. Crystals were obtained in similar conditions and diffracted to similar resolution. Their SAD structure were determined solely relying on anomalous scattering from SeO_4. One structure was determined from the peak energy and the second from the high energy remote.

Keywords: protein, phasing, selenium

MS.23.5

Acta Cryst. (2008). A**64**, C48−49

Dynamic light scattering in protein crystallization: Analysis and optimization

Christian Betzel[1], Karsten Dierks[2], Arne Meyer[1], Howard Einspahr[3]
[1]University of Hamburg, Chemistry, Institute of Biuochemistry and Molecularbiology c/o DESY, Build. 22, Notkestrasse 85, Hamburg, Hamburg, 22603, Germany, [2]PLS Design, Hamburg, Germany, Eichenstrasse 42, 20255 Hamburg, Germany, [3]Grenn Avenue 67, Lawrenceville, NJ 08648, USA, E-mail：Betzel@unisgi1.desy.de

We developed a combined imaging and dynamic light scattering (DLS) system for routine measurements in droplets of multi-well plates as well as in gel tubes used for protein crystallization. The system is of high value for rapid identification of good crystallization conditions. Today automated methods to crystallize macromolecules are widely used and can easily generate thousands of crystallization droplets. Nevertheless the evaluation of crystallization experiments to find optimal growth conditions remains a bottleneck. Therefore we have investigated methods to improve the process of evaluating results and finding crystal growth conditions. One method is DLS, the second is the use of combined white/UV illumination for determination of whether crystal-like objects are biomolecular and identification of crystals in crystallisation set-ups. Up to now, it has been impossible to determine the particle size directly in protein solution droplets because of size and configuration constraints. We have developed a CCD camera-based imaging instrument and combined a laser source and a detector to perform DLS measurements in situ. The plate-screening system allows to monitor and evaluate the entire crystallization process in an automated way. For example the stages of nucleation and the progress of crystal growth without disrupting the course of equilibration can be analyzed. The data provide information to understand in greater detail the process of crystal initiation and growth and will allow further optimisation, thereby leading to better crystals. Finally we will also describe a method to support the identification of protein crystals, exploiting the fact that most proteins and other biomolecules fluoresce when illuminated with UV light.

Keywords: crystalliztion process, dynamic light scattering, instrumentation and software

MS.24.1

Acta Cryst. (2008). A**64**, C49

Watching photo-induced dynamics with picosecond time-resolved X-ray diffraction

Shin-ichi Adachi[1,2]
[1]Photon Factory, KEK, 1-1 Oho, Tsukuba, Ibaraki, 305-0801, Japan, [2]Non-Equilibrium Dynamics Project, ERATO/JST, 1-1 Oho, Tsukuba, Ibaraki, 305-0801, Japan, E-mail：shinichi.adachi@kek.jp

Picosecond time-resolved X-ray experiments using synchrotron radiation sources are becoming general and powerful tools to explore structural dynamics of condensed matters in material and biological sciences. The beam line NW14A is a newly constructed undulator beam line for 100-ps time-resolved X-ray experiments at the Photon Factory Advanced Ring, KEK [1]. This beam line was designed to conduct a wide variety of time-resolved X-ray measurements, such as time-resolved X-ray diffraction, scattering and absorption. The beam line has been operational for two years, and current status of the beam line and scientific activities utilizing shock-induced lattice deformation [2] will be presented.

Reference:
[1] Nozawa et al. (2007) J. Synchrotron Rad. 14, 313.
[2] Ichiyanagi et al. (2007) Appl. Phys. Lett. 91, 231918.

Keywords: time-resolved diffraction, synchrotron X-ray instrumentation, structural dynamics

MS.24.2

Acta Cryst. (2008). A**64**, C49

Photo-crystallographic studies of dimerisation processes: From picoseconds to hours transformation

Joerg Hallmann, Gerhard Busse, Jav Davaasambuu, Simone Techert
Max Planck Institute for biophysical Chemistry, Am Fassberg 11, Goettingen, Niedersachsen, 37070, Germany, E-mail：jhallma1@gwdg.de

Though photo-induced solid state reactions are known since over hundred years, the structural mechanism underlying photo-induced solild state reactions have not been explored yet to a sufficient stage. In the following contribution we will present photo-crystallographic studies on [2+2] photodimerisation reactions of cinnamic acid derivatives. Common for all the investigated systems is the homogeneous character of the reaction (single crystal to single crystal transformation). We will report on high resolution photo-crystallographic studies / electron density studies monitoring the structural mechanism of the seconds to hours photodimerisation processes - and how to speed up these processes which can then only be investigated by ultrafast time-resolved crystallography (picosecond time resolution).

[1] G. Busse, Th. Tschentscher, A. Plech, M. Wulff, B. Frederichs & S. Techert, Faraday Discuss. 122 105117 (2002)
[2] J. Davaasambuu, S. Techert, J. Physics D: Appl. Physics 38, A204 − A207 (2005)
[3] J. Davasaambuu, G. Busse, S. Techert, , J. Phys. Chem. A 110 (Juergen Troe Festschrift), 3261-3265 (2006).

Keywords: photodimerization, time-resolved crystallography, organic crystals

MS.24.3

Acta Cryst. (2008). A**64**, C49−50

Picosecond and femtosecond X-ray absorption studies of the photoinduced spin change in Fe-complexes

Majed Chergui[1], Ch. Bressler[1], C. Milne[1], V.T. Pham[1], A. ElNahhas[1], R. M. van der Veen[1,2], S. Johnson[2], P. Beaud[2], D. Grolimund[2], M. Kaiser[2], C. N. Borca[2], G. Ingold[2], R. Abela[2]
[1]EPFL, SB-ISIC-LSU, BSP 427, Lausanne, Vaud, 1015, Switzerland, [2]Paul Scherrer Institut, E-mail：majed.chergui@epfl.ch

Because of close-lying low spin (LS) singlet and high spin (HS) quintet states, molecular FeII-complexes are particularly interesting in relation to the spin cross-over (SCO) phenomenon, where conversion from the LS ground state to the HS excited state (or the reverse) can be induced either by temperature, pressure or light. Several steady-state and ultrafast studies of the light-induced SCO have been carried out by optical spectroscopies, concluding that population is funneled to the HS state in < 1 ps, through intermediate singlet and triplet metal-centred (MC) states upon excitation into the singlet metal-to-ligand-charge-transfer state. However, the pathways of this cascade remain unclear, in part because the intermediate and final states are optically silent. In addition, depending on the ligand, the lifetime of the HS state spans several decades, and the origin of this large variation is unclear. In $[FeII(bpy)_3]^{2+}$ at room temperature, the HS lifetime if 660 ps. Using X-ray absorption spectroscopy (XAS) studies with 50-100 ps resolution, we found that the Fe-N bond elongates by 0.2 Å in the HS state. This elongation is similar in Fe-complexes having much longer HS lifetimes, leading us to conclude that the structure of the HS spin does not determine its lifetime, but rather it is its energetics that does. Our results show important spectral changes between the LS and HS states in the X-ray Absorption Near-Edge Structure (XANES) of the Fe K-edge.